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Published in:
Ibis

DOI:
10.1111/j.1474-919X.2008.00891.x

Ibis (2009), **151**, 171–180

Biometric sex discrimination is unreliable when sexual dimorphism varies within and between years: an example in Eurasian Oystercatchers *Haematopus ostralegus*

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Molecular sexing of birds has been possible for over a decade, but for practical reasons many studies still use biometric data for sex discrimination. In some species, the sexes are easy to distinguish but sexual dimorphism is often more subtle, requiring the use of statistical analyses of biometric measurements to discriminate sexes. These models are usually parameterized and validated using data from a limited number of sites and years. However, the resulting discriminant functions are often applied to other populations and periods. A crucial, but usually untested, assumption is that sexual dimorphism does not vary in time and space. Here we illustrate the consequences of violation of this assumption in Eurasian Oystercatchers *Haematopus ostralegus*, a species for which most studies have relied on biometric sexing. Using biometric data from captures of known-sex birds, we show that sexual dimorphism varied substantially in time and even reversed in some months and years. Furthermore, some biometric traits used in sexing changed gradually over time, causing a reduction in sexual dimorphism. We show that the consequences of this variation on sex discrimination in Oystercatchers are subtle and easily overlooked, but can result in inaccurate and strongly male- or female-biased sex-ratio estimates. We recommend that biometric sexing should be avoided in Oystercatchers unless specific calibration for each month, year and area is carried out. This recommendation also applies to other species where biometric traits may depend on environmental conditions. We argue that this condition might apply to many bird species and therefore advise caution when interpreting results based on biometric sex discrimination.

Keywords: bill morphology, environmental variation, feeding ecology, sex ratio bias, shellfish.

Ecology and behaviour often differ between the sexes. Being able to distinguish the sexes is therefore an important methodological issue. In many species, the sexes cannot be distinguished with certainty by human observers in the field. Although such species may seem sexually monomorphic at first sight, the sexes are usually not identical because small but measurable sexual dimorphisms in biometry exist.

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Reliable molecular techniques to determine the sex of these animals have been available for over 15 years (Griffiths & Tiwari 1993). Nonetheless, sex discrimination is still often based on statistical analyses of sexual dimorphism in morphometric measurements. In 2007, for example, at least 20 studies were published describing new biometric sex discrimination models for a range of bird species, and a multitude of studies have used existing biometric models to sex individuals (ISI Web of Science). Biometry is still widely used to sex individuals for at least three

reasons. First, molecular sexing of animals is often practically problematic. Extracting blood or tissue from an animal requires training and a licence, and analysis of such samples requires significant resources. Secondly, most long-term studies started when taking blood samples was not common practice. Thirdly, in many species, sex discrimination based on biometrics is thought to be reliable, or at least reliable enough for the scientific questions being addressed.

Biometric sex discrimination models are parameterized and validated using measurements of known-sex individuals from usually one or a few sites or years. Once published, these formulae are commonly used for sex discrimination in other populations or years. A crucial assumption of this approach is that sexual dimorphism is fixed (environment-independent). However, sexual dimorphism may differ between populations (Evans *et al.* 1993, van Franeker & ter Braak 1993, Zwarts *et al.* 1996). Geographical variation in sexual dimorphism may be caused by different selection pressures acting on these traits through spatial environmental variation. A consequence of geographical variation is that different discriminant functions must be developed for each population, requiring large numbers of known-sex animals from different areas (determined by DNA analysis or dissection).

Although the problem of spatial environmental variation in sexual dimorphism is generally recognized in the literature, the problem of temporal environmental variation is not. In environments that change over time, natural and sexual selection might cause morphological traits to change within and between years. Furthermore, the magnitude of morphological changes might differ between the sexes, causing a change in sexual dimorphism. Temporal changes in sexual dimorphisms in biometry can therefore affect the predictive value of biometric sex-discrimination methods. Here, we illustrate the consequences of temporal and geographical environmental variation for the reliability of sex-discrimination methods using data from a 24-year population study on Eurasian Oystercatchers *Haematopus ostralegus* at a single site, and data from seven different sites in a single year.

METHODS

Sex-discriminant functions

Various discriminant functions have been developed to determine the sex of Eurasian Oystercatchers (Heppleston & Kerridge 1970, Hulscher 1985, Durell *et al.* 1993, Lambeck *et al.* 1995, Zwarts *et al.* 1996). A discriminant function is a linear combination of one or several morphometric variables that maximizes the statistical distinction between samples of individuals of known sex. This function is then used to predict the sex of individuals for which the sex is not known (Sokal & Rohlf 1994). In this study, we assess the performance of two commonly used discriminant functions for Eurasian Oystercatchers derived by Zwarts *et al.* (1996). We chose to test discriminant functions from this particular study because they were parameterized using a large set of known-sex birds ($n > 2500$) from the same geographical area as the present study. Other studies have used slightly different discriminant functions or parameterizations thereof, but are typically based on the same biometric traits.

The first discriminant function we tested was selected for its simplicity and its wide use. This function, which we call D_R, uses the ratio between the length and depth of an Oystercatcher's bill (Fig. 1). Because female Oystercatchers have on average longer and narrower bills than males, the discriminant function defines a critical bill length-to-depth ratio above which individuals are most likely to be female. Using a large dataset of known-sex birds, Zwarts *et al.* (1996) showed that this discriminant function correctly classified the sex of 87% of all birds in their sample (using a critical bill length-to-depth ratio of 7.13).

The second discriminant function tested was selected because it was the best predictor of the sex of Oystercatchers in the study of Zwarts *et al.* (1996).

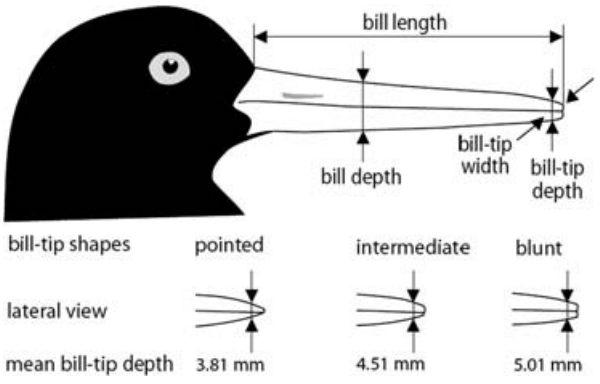

Figure 1. Measurement and qualification of bill morphology. Bill length (from tip to back edge of frontal shield) and bill depth (at the gonys) were measured quantitatively, and the shape of the bill-tip was classified qualitatively (i.e. pointed, intermediate, blunt). The shape of the bill-tip is mainly determined by the depth and width of the bill-tip, but these quantitative measurements were not always taken. Figure adapted from Zwarts *et al.* (1996).

This discriminant function, which we call D_S, includes four biometric measures: body mass and three measures of bill morphology. Bill length and depth were measured quantitatively, whereas the shape of the bill was classified qualitatively (Fig. 1). The bill of Oystercatchers has a variety of distinct shapes, caused by differences in feeding ecology (Swennen *et al.* 1983, Hulscher 1985, Durell *et al.* 1993). The tip of the bill of Oystercatchers feeding on hard-shelled shellfish abrades at a high rate and consequently has a blunt shape, whereas that of Oystercatchers feeding on worms remains pointed. A mixed diet of soft-shelled clams and worms results in an intermediate bill shape. The discriminant function D_S has the following form:

$$D_S = a_S + b_S \times BodyMass + c_S \times BillLength + d_S \times BillDepth$$

where the values of parameters a_S, b_S, c_S and d_S are dependent on the bill shape (S = pointed, intermediate, or blunt). For example, for birds with a blunt bill, $D_S = -4.81 + 0.006BodyMass + 0.30 \times BillLength - 1.60 \times BillDepth$; other parameter values are given in Table 3 in Zwarts *et al.* (1996). When the value of D_S is larger than zero the individual is classified as a female, when negative as a male. Zwarts *et al.* (1996) found that the D_S discriminant function correctly classified the sex of 93% of all birds in their dataset. They also showed that due to age-related changes in morphometric traits, different discriminant functions have to be used for yearlings and (sub)adults. Furthermore, they showed that because of geographic variation in morphometric traits, different discriminant functions have to be used for coastal and inland birds (sites 1–3 vs. sites 4–5 in Fig. 2).

Data collection

We assessed the performance of the two discriminant functions mentioned above in two new datasets of measurements of Eurasian Oystercatchers. We limited analyses to data from adult birds, to remove age-dependent variation. In addition, we limited analysis to coastal sites (i.e. excluded inland sites) to assess whether geographic variation exists between coastal sites within the same estuary, as Zwarts *et al.* (1996) has already shown that geographical variation exists between coastal and inland sites. The first dataset was used to assess the importance of temporal variation, within as well as between years. This dataset comprises adults caught at a single coastal population, on the Dutch Wadden Sea island of Schiermonnikoog (site 5 in Fig. 2). From 1983 to 2006, we made 1487 catches of 714 different individuals between March and August. The second dataset was used to assess the importance of geographical variation. This dataset comprised 206 different adults captured in a single winter (November 2002–March 2003) at seven coastal sites in the Dutch Wadden Sea (sites 5–11 in Fig. 2).

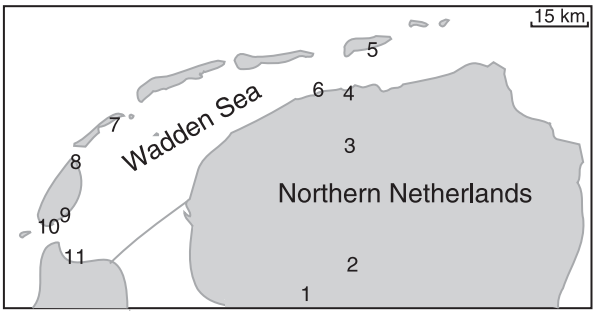

Figure 2. The catching sites in northern Netherlands where Oystercatchers were sampled: 1 South Friesland, 2 Drachten, 3 North Friesland, 4 Paesens, 5 Schiermonnikoog, 6 Wierum, 7 Vlieland, 8 Texel-Cocksdorp, 9 Texel-NIOZ, 10 Texel-Mokbaai, 11 Balgzand. The study of Zwarts *et al.* (1996) sampled birds from sites 1–5, the first dataset from our study comprised birds from site 5, the second dataset from our study comprised birds from site 5–11.

Birds were caught on the nest with a walk-in cage in the months of May, June and July; birds caught in other months were caught on communal roosts using cannon and mist nets. The sex of individuals was determined either directly by DNA analysis (62%) or indirectly when their nesting partner was sexed using DNA analysis (38%). Following Zwarts *et al.* (1996), we measured body mass, bill length, bill depth, and bill-tip shape. In the first dataset, the vast majority of measurements were taken by 10 researchers involved in the study for several years. To assess the amount of measurement error, two observers independently measured the same captured bird in 87 cases. In the second dataset, all birds were measured by the same observer (K.O.).

Data analysis

Generalized linear mixed models (GLMM) were used to estimate the average annual and monthly values for each biometric trait. For the approximately normally distributed dependent variables relating to body mass, bill length and bill depth, we used the identity link function and specified a Gaussian error distribution. For the analysis of the categorical bill shape variable, we combined the pointed and

intermediate bill shapes in one group (i.e. non-blunt) and estimated the probability of having a blunt bill using a logit link function and a binomial error distribution. The sex of a bird, and the year and month of catching were included as categorical fixed effects, as well as the interaction between sex*year and sex*month. The identity of the observer was added as a random effect to assess any systematic variation between observers in their measurements. Furthermore, the identity of a bird was added as a random effect to account for the fact that many individuals feature more than once in the first dataset. Temporal trends in annual/monthly values were analysed using the annual estimates derived from the above models as normally distributed response variable and year/month as a linear covariate. All models were analysed using MLwiN 2.0 or SPSS 15.0.

RESULTS

Measurement error, observer effects and individual variation

Measurement error of all four biometric traits appeared small as the measurements by two observers of the same captured birds were highly correlated ($r > 0.90$ for all four traits, $n = 87$ birds). Furthermore, there were no indications of strong systematic differences between observers as observer identity did not explain significant variance when entered as a random effect into models of the biometric traits (GLMM: $P > 0.25$ for all four traits; $n = 1487$). In all analyses, the individual random effect (i.e. bird identity) was highly significant (GLMM: $P < 0.01$ for all four traits; $n = 1487$). Most of the variation in body mass (75%), bill length (91%) and bill depth (70%) was explained by among-individual variation. In contrast, most of the variation in bill shape was due to within-individual variation (81%), suggesting bill shape is the most plastic trait.

Sexual dimorphism

Oystercatchers in the first dataset were sexually dimorphic in all four traits used in the discriminant functions of Zwarts *et al.* (1996), in the sense that there was a statistically significant difference between the sexes, but distributions overlapped substantially (Fig. 3). Nonetheless, as with previous studies, the average female in our population was heavier and had a longer and more slender bill than the average male. Furthermore, the average female was more likely to have a bill shape associated with feeding on worms (pointed or intermediate), whereas males more often had bill shapes associated with feeding on shellfish (blunt). This confirms that these measures can in principle be used to discriminate statistically between male and female Oystercatchers.

Temporal variation

The four biometric traits used in sex discrimination and the degree of sexual dimorphism varied significantly between years. There was substantial between-year variation in the average measurements of both males and females. For example, average body mass of males in one year was as high as the average body mass of females in another year (Fig. 4a). This annual variation was not caused by sampling variance, as standard errors of the annual means were small and were based on an average of 31 individuals of each sex per year (test of year effects: $\chi^2_{23,1456} = 115.5$, $P < 0.001$). In some years, the sexual dimorphism in bill depth between males and females was even reversed (Fig. 4c). Average values of morphological traits also changed systematically over time. For example, body mass decreased over the 24 years of data and consequently both sexes became more masculine with respect to this trait (Fig. 4a; $F_{1,45} = 5.14$, $P = 0.028$). Conversely, the blunt-bill type virtually disappeared over the years and thus both sexes became more feminine with respect to this trait (Fig. 4d; $F_{1,45} = 31.61$, $P < 0.001$). The rate of change in bill shape over the years also differed between the sexes (ANOVA year*sex $F_{1,44} = 7.29$, $P = 0.010$), indicating that the sexual dimorphism in bill shape became smaller over time (Fig. 4d). The only biometric trait that remained relatively constant over time was the bill length (Fig. 4b).

Temporal patterns in the biometric traits showed that the degree of sexual dimorphism also varied systematically within years. Body mass peaked just before the start of the breeding season in May–June (Fig. 4e). Furthermore, blunt bills became less common across the course of the breeding season (Fig. 4h). The direction of the sexual dimorphism was also not fixed within a year. In most months, females had a narrower bill than males; however, this sexual dimorphism was reversed for birds caught in March (Fig. 4g).

Using the two discriminant functions of Zwarts *et al.* (1996), we classified all individuals as either a male or female, and subsequently compared their predicted sex with their known-sex. Overall, the discriminant function that used the bill length-to-depth ratio

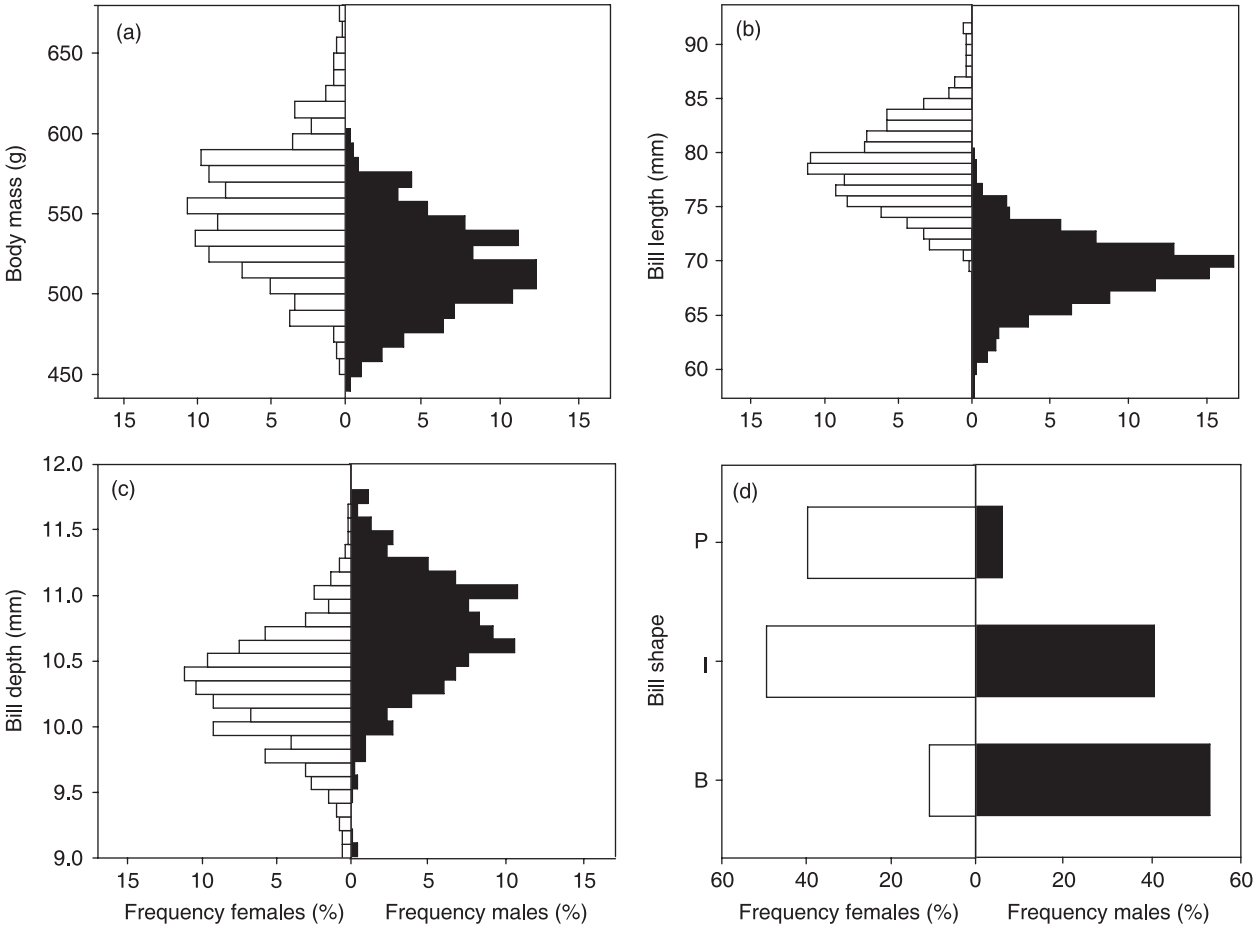

Figure 3. Frequency distributions of biometric measurements of females (white) and males (black) that were used for sex determination: (a) body mass, (b) bill length, (c) bill depth, and (d) bill shape (*P* = pointed, I = intermediate, B = blunt). Data from birds caught in site 5 (see Fig. 2) in the months of March–August from 1983–2006 ($n = 1487$).

(D_R) sexed 93.8% of all birds correctly, higher than the accuracy of 87.3% reported by Zwarts *et al.* (1996). However, the accuracy was higher for known-sex males (95.9%) than for females (91.5%), showing that the method is biased towards classification as male in the first dataset (as females are more often misclassified as males than *vice versa*). Overall, the discriminant function that used four different biometric traits (D_S) sexed 90.0% of all birds correctly, lower than the accuracy of 93.2% reported by Zwarts *et al.* (1996). Again, the accuracy was higher for males (92.9%) than for females (86.8%), showing that this method is also biased towards classification as male.

As a result of the temporal variation in biometrics (Fig. 4), the D_R and D_S discriminant functions also varied considerably between and within years (Fig. 5), which caused the accuracy of both discriminant functions to vary systematically between and within years. In most years, the accuracy was as high as previously reported (> 90%) and misclassifications were equally common for both sexes (Fig. 5). However, in a number of years the accuracy of the sex estimates was low (as low as 65% in 2003). Furthermore, in these years the discriminant functions were also extremely biased, as virtually all phenotypes were classified as males (i.e. most males were correctly sexed, and the majority of females incorrectly sexed as male). Females were mostly misclassified in 2002–04 because of their high (masculine) bill depth in those years (Fig. 4c). The reason D_S performed worse than D_R in 2002–04 is that the formula of D_S puts more weight on bill depth than D_R (Zwarts *et al.* 1996). D_S also misclassified most females in 1999, probably because of the low and variable body mass of females in that year (Fig. 4a). Also within years,

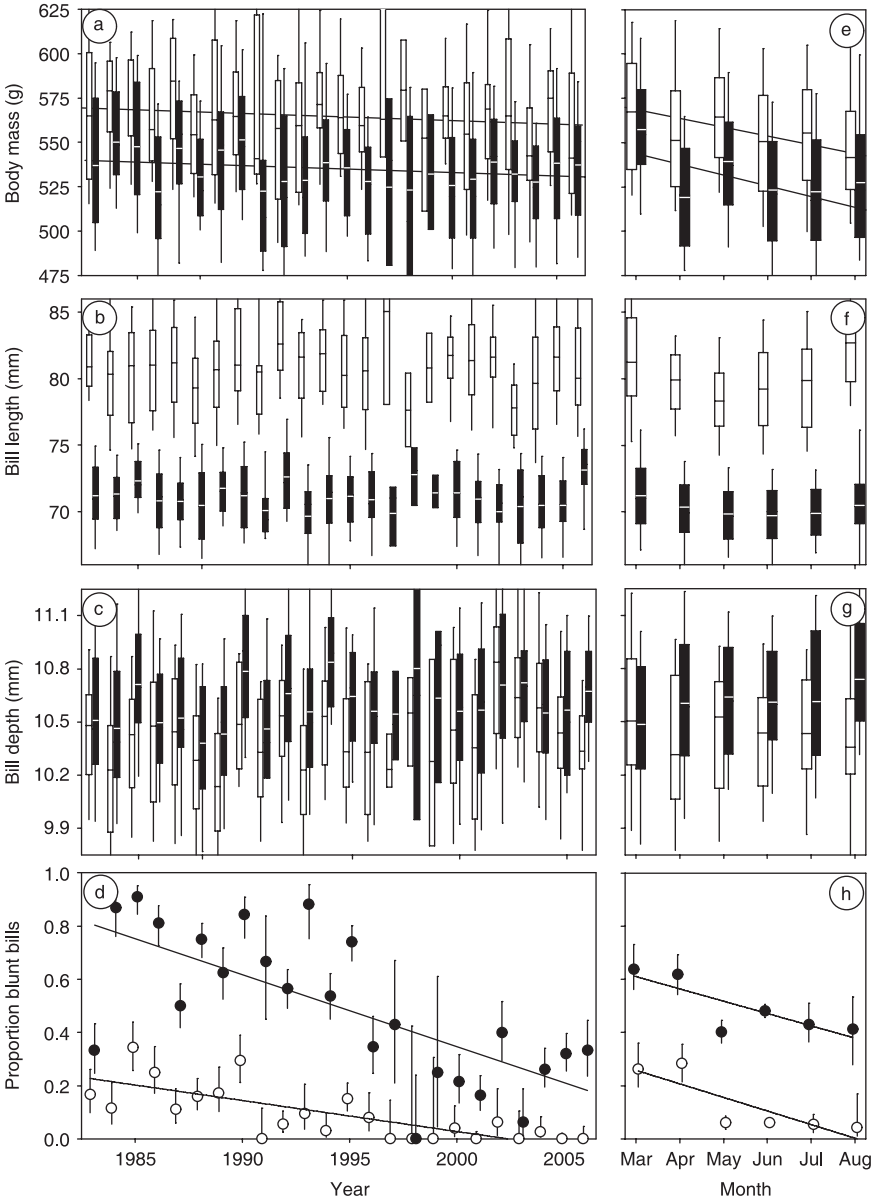

Figure 4. Between- and within-year variation in biometric measurements used for sex discrimination. Presented are mean annual (a–d) and monthly (e–h) values for females (white) and males (black). Trend lines are used for regression coefficients significantly different from zero at the 5% level (see text). In (a–c) and (e–g), box-plots are based on means with quartiles and error bars based on 10% and 90% confidence intervals. In (d and h), circles are based on means with error bars based on standard error. Annual values were adjusted for the month of capture, whereas monthly values were adjusted for the year of capture. Data from site 5 (see Fig. 2; $n = 1487$).

both methods were more biased in some months than in others (Fig. 5). In particular, females caught in March were misclassified, due to the reversal of sexual dimorphism in bill depth in that month (Fig. 4).

The biological consequences of the bias in sex estimates in specific years or months can be severe. The discriminant functions predicted that the sex ratio of birds caught in 2002, 2003 and 2004 was strongly male-skewed (D_R: 71%, 80%, 63%; D_S: 71%, 88%, 72%, respectively), while the sex ratio based on DNA analysis was closer to equality (59%, 61%, 55% respectively). Among birds caught in March both discriminant functions also predicted a stronger male-skew in the adult sex ratio (D_R: 56%; D_S: 66%) than was actually observed (52%).

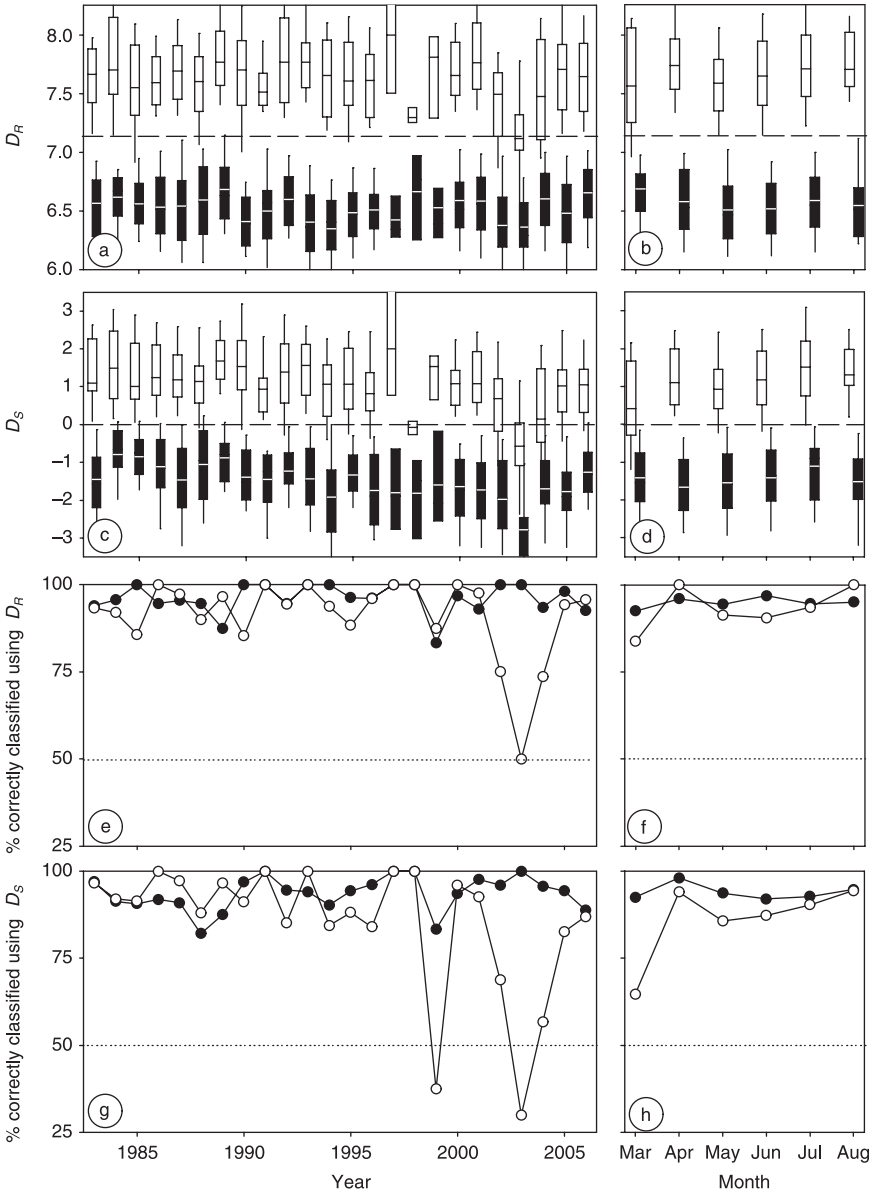

Figure 5. Between- and within-year variation in (a–d) discriminant scores and (e–h) percentages of correctly classified females (white) and males (black). In (a–d), box-plots are based on means with quartiles and error bars based on 10% and 90% confidence intervals. The critical D_R and D_S values of Zwarts *et al.* (1996) are depicted with a dashed line. In (e–h), the performance of D_R and D_S to sex males and females correctly is plotted for each year and month. Data from site 5 (see Fig. 2; $n = 1487$) with annual values based on 11–60 individuals per sex (except for 1997–99: 5–10 per sex) and monthly values based on 18–451 individuals per sex.

Geographical variation

To assess the importance of geographical variation between coastal sites, we investigated whether the predictive power of the discriminant functions (D_R and D_S) differed between populations in the second (winter) dataset. As expected, the sex of a bird was related to both D_R and D_S (logistic regression: D_R $\chi^2_{1,204} = 831.8$, $P < 0.001$; D_S $\chi^2_{1,204} = 18.4$, $P < 0.001$). However, the relationship between the discriminant functions and the known-sex of a bird was very different from that predicted by Zwarts *et al.* (1996) (Fig. 6; curve 2 vs. curve Z). Applying the discriminant functions of Zwarts *et al.* (1996) on the second dataset would classify most females (> 90%) correctly but the majority of males (> 50%) incorrectly.

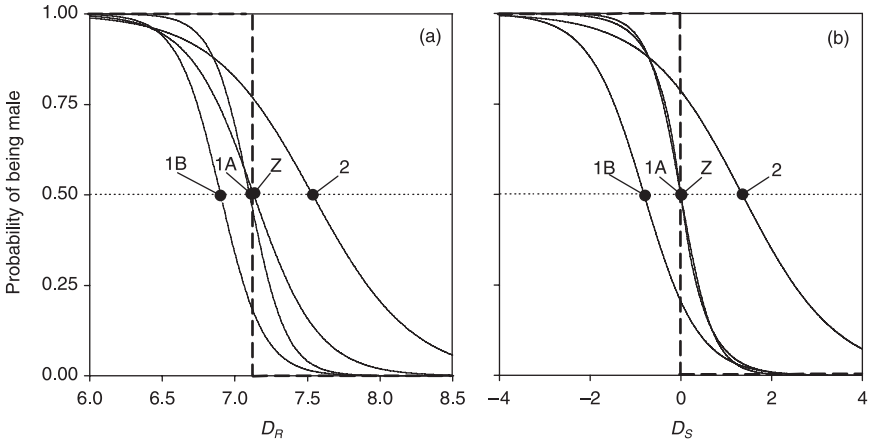

Figure 6. The relationship between the probability of being a male and the discriminant score (a) D_R (i.e. bill length-to-depth ratio) and (b) D_S for different datasets. Labels refer to different datasets: 1A: subset of the first dataset, March–August 1983–2001, site 5 in Fig. 2 ($n = 1180$); 1B: subset of the first dataset, March–August 2002–03, site 5 ($n = 68$); 2: second dataset, December 2002–March 2003, sites 5–11 ($n = 206$); Z: data of Zwarts *et al.* (1996), January–December 1973–93, sites 1–5 ($n = 2729$). The point where the curves cross the horizontal dotted line are the critical values that best discriminate between the sexes in each dataset. This critical value is translated in a discrete decision rule, as illustrated by the dashed line for the Zwarts *et al.* (1996) dataset.

Thus the discriminant functions derived by Zwarts *et al.* (1996) were strongly female-biased in the second (winter) dataset, whereas they were male-biased in the first dataset in the preceding and following summer of that same year (Fig. 6; curve 1B vs. curve 2).

There was no significant between-site variation in the relationship between sex and D_R (logistic regression: sites $\chi^2_{6,198} = 8.57$, $P = 0.20$), or between sex and D_S (sites $\chi^2_{6,198} = 7.56$, $P = 0.27$). Furthermore, the slope of the relationship between the sex of a bird and the discriminant functions also did not differ among sites (logistic regression: D_R*site $\chi^2_{6,192} = 10.06$, $P = 0.12$; D_S*site $\chi^2_{6,192} = 5.88$, $P = 0.44$). Thus there was no evidence that the sexual dimorphism as quantified using the discriminant scores differed among coastal sites within the Dutch Wadden Sea.

DISCUSSION

Temporal variation

The results suggest that large temporal variation (within and between years) can exist in morphometric traits that are used to discriminate the sexes. Whether the morphological changes over the 24 years of study were the result of within-individual changes (i.e. phenotypic plasticity) or between-individual changes (i.e. selection) will be assessed elsewhere. Independent of the mechanism of morphological change, many of the observed changes are likely to have resulted from temporal environmental variation. For example, blunt bills, associated with a shellfish diet, became less common towards the breeding season (Fig. 4h), consistent with the observation that Oystercatchers switch to feeding on worms in summer because these are more readily available than in winter (Bunskoeke *et al.* 1996). Some morphological changes may be the result of human-induced environmental change. Mechanical shell-fisheries have greatly reduced shellfish stocks in the entire Dutch Wadden Sea (Beukema & Cadée 1996, Piersma *et al.* 2001, Ens 2006) and regional variation in the legal access of shellfish fisheries has been shown to be associated with the shape of the bill tip (Verhulst *et al.* 2004). Also within our main study site (site 5), the observed long-term decline in body mass and the disappearance of blunt bill types were correlated with declining local shellfish abundance over the 24-year period (van de Pol 2006).

Studies that sex birds using biometry assume that the sexual dimorphism in these traits is fixed in time. This assumption is implicit, as discriminant functions are typically parameterized using known-sex birds from a few months or years, and these functions are subsequently used to determine the sex of unknown birds in other months and years. However, biometry often depends on the environment, which generally changes both seasonally and over the years. This study demonstrates that the consequences of temporal variation in biometric traits on sex discrimination can be subtle and remain unnoticed for many years.

On average, the accuracy of the discriminant functions was quite acceptable (> 90%), but in a few months and years estimates were very imprecise (e.g. 65% in 2003) and severely biased (e.g. 30% correct for females and 100% correct for males in 2003). Strikingly, the methodological bias was not always in the same direction, as discriminant functions were male-biased in the summer (first) dataset, but female-biased in the winter (second) dataset (Fig. 6). Such methodological biases can be problematic for many scientific questions, for example for the study of sex ratios.

Unbiased estimates of adult sex ratios are important for population viability analyses, as a skewed sex ratio directly affects the effective population size and demographic stochasticity (Lande *et al.* 2003). Several studies suggested that Oystercatcher populations have a skewed adult population sex ratio, with a skew towards males in the UK (Durell & Goss-Custard 1996, Durell & Atkinson 2004, Durell 2006) and towards females in the Netherlands (Verhulst *et al.* 2004). As all these studies have used bill morphology to discriminate the sex of Oystercatchers, it seems desirable to assess whether these reported sex ratio skews are methodological artefacts caused by temporal (or geographic) variation in bill morphology. For example, Verhulst *et al.* (2004) used the D_R discriminant function of Zwarts *et al.* (1996) (curve Z in Fig 6a) to arrive at their estimated sex ratio of 44.6% males. The dataset of Verhulst *et al.* is very similar to the second dataset used in this study, as it consisted of 518 birds caught in the winter of 2001 at seven coastal sites, of which several sites were the same as in the second dataset. When re-analysing the data of Verhulst *et al.* (2004) using the discriminant function derived from the second dataset (curve 2 in Fig. 6a), the estimated sex ratio changes to being strongly male-skewed (68.0% males) instead of the published female bias (44.6% males). Thus, the various estimates of Oystercatcher adult sex ratios can be highly dependent on the discriminant function used, and DNA-based sex discrimination is required to be certain about the real population sex ratio (assuming catches reflect a random sample from the population).

Geographical variation

Although temporal variation in sexual dimorphism was large, we found no strong evidence for geographical variation between coastal sites within a single Dutch estuary. The latter is in agreement with Zwarts *et al.* (1996), who reported only geographical variation between Dutch coastal and inland sites. Nonetheless, geographic variation between coastal sites does exist at a larger spatial scale, as Zwarts *et al.* (1996) showed that a critical D_R of 7.1 best separated the sexes in the Dutch Wadden Sea, whereas Heppleston and Kerridge (1970) reported a critical ratio of 6.7 and 6.3 for two British estuaries. Applying the discriminant values of these British estuaries to our study would have misclassified 15–38% of all birds (see Figs 5a,b and 6a), suggesting that discriminant functions to sex Eurasian Oystercatchers in one estuary cannot simply be applied to other estuaries.

General implications

The use of morphological traits for sex discrimination is still widely accepted in the ornithological literature, and body mass and bill morphology are commonly used in discriminant functions (see references in Introduction). However, morphological traits can exhibit phenotypic plasticity, and directional phenotypic selection on such traits may not be uncommon (Kingsolver *et al.* 2001). Traits like body mass and bill morphology might be especially sensitive to environmental change. In most species, body mass is strongly associated with nutrient storage and thus expected to react strongly on environmental variation in food abundance. Similarly, the association between food availability and bill morphology is a well-known example of how environmental change can result in (evolutionary) morphological change (e.g. Grant 1999, Badyaev *et al.* 2000, Marquiss & Rae 2002; see also Swennen *et al.* 1983, Hulscher 1985). Thus, it seems quite likely that the sexual dimorphism in these biometric traits might not be independent of temporal environmental variation in other species. This might be particularly true for other oystercatchers, as all 12 closely related oystercatcher species are only slightly sexually dimorphic (Hockey 1996), have a bill morphology closely adapted to their feeding behaviour and can exhibit substantial temporal variation in body mass and bill morphology (Baker 1974, Hockey 1981, Hulscher 1985, Lauro & Nol 1995, Hockey 1996). Therefore, it is important either to assess at regular intervals whether the chosen biometric sex discrimination method can be as generally applied as is often implicitly assumed, or to apply DNA-based sex-discrimination methods when possible. In cases where this is not possible, for example because data were collected in the pre-blood-sampling era or because data are already published,

the interpretation of these (published) results might require caution.

We would like to thank Natuurmonumenten for allowing us to work in the national park Schiermonnikoog. We thank Rudi Drent for continuous support, and all the people who contributed to the fieldwork, especially Jan Hulscher, Marcel Kersten, Dik Heg and Leo Bruinzeel. Lyanne Brouwer, Olivier Duriez and an anonymous reviewer provided valuable comments on the manuscript. M.v.d.P. was supported by a Rubicon fellowship from the Netherlands Organization for Scientific Research (NWO; grant 825.06.032). The Technology Foundation STW (code GBI 4804), applied science division of NWO and the technology programme of the Ministry of Economic Affairs supported the research. S.V. was supported by a VICI-grant from NWO.

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Received 4 June 2008;
revision accepted 10 September 2008.